Folia Microbiol. 44 (4), 449–453 (1999) http://www.biomed.cas.cz/mbu/folia/

Comparative Genetic Study of Group B Streptococcal Strains of Human and Bovine Origin*

A. DMITRIEV[a]**, Ľ. TKÁČIKOVÁ[b], A. SUVOROV[a], M. KANTÍKOVÁ[c], I. MIKULA[d], A. TOTOLYAN[a]

[a]*Institute of Experimental Medicine, 197 376 St. Petersburg, Russia*
fax +007 812 234 0542 e-mail admitriev@actor.ru
[b]*University of Veterinary Medicine, 041 81 Košice, Slovakia*
fax +421 96 632 3666 e-mail mikula@uvm.sk
[c]*State Veterinary Institute, 026 01 Dolný Kubín, Slovakia*
fax +421 845 586 4010 e-mail riaditelsvudk@isternet.sk
[d]*Neuroimmunological Institute, Slovak Academy of Sciences, 842 46 Bratislava, Slovakia*

Received 17 October 1999

ABSTRACT. The presence and restriction fragment length polymorphism (RFLP) of DNA fragments hybridizing with virulence and "house keeping" gene probes were analyzed for 87 group B streptococcal (GBS) strains of human and bovine origin. Most characteristics obtained for bovine strains were similar when compared with those for human strains. The most significant degree of RFLP was discovered for the sizes of *Hin*dIII fragments containing *bca* gene. Human GBS strains with *bac* gene, encoding β antigen with IgA binding capacity, were characterized by almost identical complex hybridization patterns with multiple gene probes. At the same time *bac* gene was not found among bovine GBS strains. Gene *scpB* that encodes C5a peptidase in all human GBS strains was detected only in 9 of 39 strains of bovine origin. These two characteristics effectively distinguished bovine GBS strains from GBS strains of human origin.

Streptococcus agalactiae (group B streptococci, GBS) are Gram-positive bacteria with the wide range of the hosts. Group B streptococci are isolated from both human and animals such as cows, cattle, pigs, nutrias *etc.* (Smola 1993). GBS, being the important human pathogen causing the pathology of pregnancy and infectious diseases of the newborns (Baker and Edwards 1990), remains actual also for veterinary practice as a causative agent of the dairy cow mastitis. There are no exact evidences for the exchange of GBS strains between animals and human. The question, if different GBS strains of animal origin can persist in human, is still open. GBS of bovine origin are usually isolated from the cow milk and probably can be transmitted to human. But at present there are no strong evidences on epidemiological identity of the strains isolated from both sources. A little is known regarding animal GBS genetics (Bentley and Leigh 1996; Denning *et al.* 1989; Forsman *et al.* 1997) and nothing is known about the distribution of virulence genes specific for human GBS strains among GBS strains isolated from the dairy cows while human GBS strains were well characterized immunologically and genetically. The present study was devoted to the comparative analysis of the genetic diversity of human and bovine GBS strains and investigation of the presence of virulence genes specific for human GBS among GBS isolated from the dairy cows.

MATERIALS AND METHODS

Thirty four GBS strains of human origin were isolated in Beijing (P.R. China), 39 strains of bovine origin were isolated from the cow milk in different regions of Slovakia. Bacteria were cultivated in Todd–Hewitt broth (*Difco*, USA) at 37 °C. All strains were identified by group B specific antiserum. IgA binding activity of the strains was determined according to Suvorov *et al.* (1997). *Hin*dIII and *Pvu*II ribosomal types of GBS strains were determined using *rrs* gene as a probe. Additionally *Hin*dIII ribotypes were determined for 14 human strains isolated in St. Petersburg (Russia). The presence of virulence and "house keeping" genes in the genome of GBS strains under study was tested by PCR and DNA–DNA hybridization. DNA probes for the investigation were prepared by labeling of the recombinant plasmids or PCR products using DNA Labeling and Detection kit (*Boehringer*, Mannheim, Germany). The other procedures were done according to (Maniatis *et al.* 1982). The sizes of DNA fragments were estimated employing computer program SEQAID.

*Presented at the *International Conference on Actual Problems in Microbiology and Immunology*, Košice (Slovakia), 13–15 October 1999.

**Present address*: University of Veterinary Medicine, 041 81 Košice, Slovakia.

RESULTS AND DISCUSSION

Thirty four Chinese human GBS strains and 30 of 39 GBS strains of bovine origin were tested by DNA–DNA hybridization for the presence of *bca*, *bac*, *scpB*, *scaA* and *glnA* genes, encoding the surface proteins — α-antigen, β-antigen, C5a peptidase, aggregation factor and glutamine synthetase potentially involved in virulence of human GBS and "house keeping" gene *pet*. *Hin*dIII and *Eco*RI endonucleases were used to digest chromosomal DNA. Genes *scaA*, *pet* and *glnA* were found in all GBS strains tested. The certain degree of RFLP was found for DNA fragments hybridizing with *glnA* and *pet* gene probes, while the sizes of *Hin*dIII fragments, containing *scaA* gene, were equal to 2.0 kb for all the strains (Table I).

Table I. Ribosomal types of GBS strains and the sizes of DNA fragments hybridizing with gene probes

Gene probe	Restriction enzyme	Human GBS strains		Bovine GBS strains	
		size, kb	number	size, kb	number
scaA	*Hin*dIII	2.0	34	2.0	30
pet	*Eco*RI	3.2	8	3.2	10
		3.8	26	3.8	20
glnA	*Eco*RI	6.5	29	6.5	10
		11.2	3	8.4	11
		12.6	2	9.7	9
bac	*Hin*dIII	3.1	15	—[b]	39
		3.0	1		
		3.2	1		
		—[a]	17		
bca	*Hin*dIII	4.2–7.7	18	4.2–7.5	18
		—[a]	16	—[b]	21
scpB	*Hin*dIII	2.6	27	2.6	9
		2.7	7	—[a]	30
rrs	*Hin*dIII	H1*	37	H1*	10
		H2*	3	H3*	14
		H3*	8	H4*	5
		—	14[c]	H5*	1

[a]Not detected. [b]Not detected including strains tested by PCR only.
[c]Including Russian strains tested additionally. *rDNA pattern.

Gene *scpB* encoding C5a peptidase was found in all human GBS strains under study. Unexpectedly this gene was detected only in 9 of 39 bovine GBS strains by DNA–DNA hybridization. The sizes of *Hin*dIII fragments hybridizing with *scpB* gene probe were equal to 2.6 or 2.7 kb for human strains and 2.6 kb for *scpB* gene positive bovine strains. Fifty one bp deletion in 3′ region of *scpB* gene of GBS strains in comparison with *scpA* gene of group A streptococci (GAS) is an important difference between *scpB* and *scpA* genes that was previously discovered (Chmouryguina *et al.* 1996). The primers

5′-ACAATGGAAGGCTCTACTGTTC-3′ and
5′-ACCTGGTGTTTGACCTGAACTA-3′

for the region around this deletion were constructed to determine the presence of 51 bp deletion in *scpB* gene of GBS strains. Thirty four Chinese and 14 Russian human GBS strains as well as 39 Slovakian bovine strains were investigated by PCR. After PCR the fragments of the expected size of 255 bp were amplified. This size was the same for all human GBS strains and *scpB* gene positive bovine GBS strains (Fig. 1) while GAS strains revealed 306 bp amplifying fragment (*data not shown*). It indicates the presence of 51 bp deletion in *scpB* gene of both human and bovine GBS strains. The presence

of 51 bp deletion in all human GBS strains could be a suitable marker for differentiation of GAS from human GBS. Interestingly 9 of 39 bovine strains (77 %) were found to be *scpB* gene negative strains while all human GBS strains possessed this gene. Probably only *scpB* gene positive GBS strains of animal origin can be infective for human.

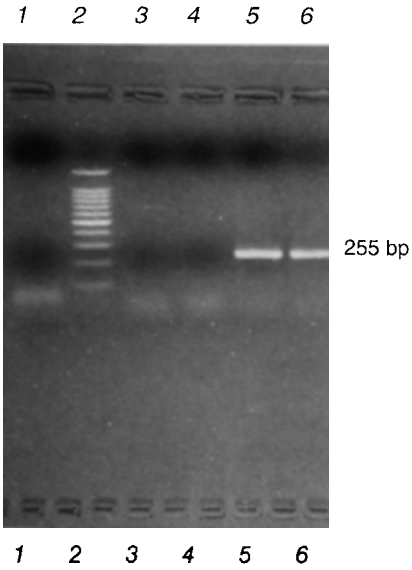

Fig. 1. Detection of *scpB* gene in group B streptococcal strains by PCR; *1* — negative control, *2* — 100 bp ladder, *3, 4* — *scpB* gene negative bovine GBS strains, *5* — *scpB* gene positive bovine GBS strain, *6* — *scpB* gene positive human GBS strain.

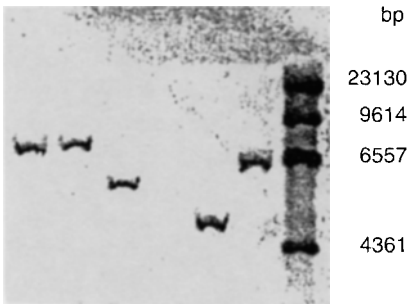

Fig. 2. Different sizes of *Hin*dIII fragments hybridizing with *bca* gene probe.

The presence of gene *bac*, encoding IgA binding β-antigen of human GBS (Heden *et al.* 1991; Jerlstrom *et al.* 1991) was investigated among 34 human strains and 39 bovine strains by hybridization or PCR. The recombinant plasmid pS1 (Suvorov *et al.* 1997) was used as a probe for DNA–DNA hybridization. Surprisingly *bac* gene was not revealed in bovine strains at all. From the other hand this gene was detected in 17 of 34 human strains tested (Table I). Recently it was proposed that the presence of *bac* gene is the genetic marker which distinguished GBS in two lineages (Dmitriev *et al.* 1998). The results of the present study probably indicate that *bac*$^+$ genetic lineage of GBS is specific for human strains though screening of a larger bovine strains collection for the presence of *bac* gene should be performed. The question if the strains of *bac*$^+$ genetic lineage can persist in animals is still open.

The presence of another virulence gene of human GBS strains — gene *bca* (Michel *et al.* 1993) — was detected by DNA–DNA hybridization and PCR. Two primers, *viz.*

5′-CAGGAGGGGAAACAACAGTAC-3′ and
5′-GTATCCTTTGATCCATCTGGATACG-3′

for *bca* gene specific repeats were constructed and after PCR analysis it was established that 18 of 39 bovine strains and 18 of 34 human strains possessed this gene. Some strains produced only 1 intensive band of the expected size of 184 bp. Other strains produced different numbers of DNA fragments that presented ladder-like structure. The size of the smallest fragment was also 184 bp. The sizes of amplified fragments were similar both for human and bovine strains. The presence of *bca* gene in the genome was confirmed by DNA–DNA hybridization. For this purpose 184 bp amplifying fragment was isolated from agarose and used as a probe. Interestingly the sizes of *Hin*dIII fragments containing *bca* gene were determined as different and varied from 4.2 to 7.5 kb for bovine strains and from 4.2 to 7.7 kb for human strains (Fig. 2). These results can be explained by the different numbers of specific repeats in *bca* gene structure (Michel *et al.* 1993).

*Hin*dIII ribosomal types of 48 human strains and 30 bovine strains were determined using *rrs* gene as a probe. *Hin*dIII enzyme was found to have unsufficient discriminative power for ribotyping. As result, 5 *Hin*dIII rDNA patterns were revealed among the strains tested (Table I). Forty seven, 3, 22, 5

and 1 strains were characterized by H1, H2, H3, H4 and H5 *Hin*dIII rDNA patterns, respectively. H1 and H3 rDNA patterns were predominant for both human and bovine GBS strains. Forty five of 48 human strains (94 %) and 24 of 30 bovine strains (80 %) belonged to ribosomal types H1 and H3. Interestingly, 5 bovine strains isolated in the same farm belonged to ribotype H4. The sizes of DNA fragments hybridizing with different gene probes were also similar for these strains. It can reflect the possible epidemiological similarity between these strains. From the other hand some strains isolated in another farm were different only in size of *Hin*dIII fragment hybridizing with *bca* gene. The predominant rDNA patterns H1 and H3 were characteristical for the strains isolated in Russia, Slovakia and China that reflects independence of *Hin*dIII GBS ribotypes of the geographic area. No correlation between origin of GBS strains and their *Hin*dIII ribosomal types was observed as well as *Hin*dIII could be used for the efficient differentiation of the strains.

Additionally, *Pvu*II ribosomal types were determined for 28 human strains. Analyzing *Pvu*II rDNA patterns it was found out that 16 of 17 human strains carrying *bac* gene belonged to the same ribotype P1. Another 12 strains tested belonged to 10 different ribotypes P1–P10. It indicates the effectiveness of *Pvu*II ribotyping for all the strains except the strains with *bac* gene. Most of other characteristics of *bac* gene positive GBS strains were also similar except the size of *Hin*dIII fragment, containing *bca* gene. So, the number of specific repeats in the structure of gene *bca* can be considered as a most suitable discriminative marker especially when other markers can not distinguish GBS strains.

DNA–DNA hybridization analysis with gene probes provided the opportunity of the simultaneous analysis of different regions of the GBS genomes by comparison of strain specific hybridization patterns. Surprisingly the hybridization patterns of GBS strains with *bac* gene were found to be almost identical. This finding leads us to the proposal of genetic relationship of the strains, carrying *bac* gene, which possibly gives the GBS strains some biological advantages. At the same time *bac* gene was not found among the bovine GBS strains. Gene *scpB* that encodes C5a peptidase in all human strains was detected only in 9 of 39 strains of bovine origin.

Table II. Genetic types of human and bovine GBS strains used (%)

Genetic type	Human	Bovine
bca$^+$ *bac*$^+$ *scpB*$^+$	50	0
bca$^+$ *bac scpB*$^+$	3	23
bca bac scpB$^+$	47	0
bca$^+$ *bac scpB*	0	26
bca bac scpB	0	51

Three genotypes, *bca*$^+$ *scpB*$^+$ *bac*, *bca*$^+$ *scpB bac* and *bca scpB bac*, were revealed among bovine GBS strains. Also three genotypes, *bca*$^+$ *scpB*$^+$ *bac*, *bca*$^+$ *scpB*$^+$ *bac*$^+$ and *bca scpB*$^+$ *bac*, were revealed among human GBS strains (Table II). Interestingly, the strains of *bca*$^+$ *scpB bac* and *bca scpB bac* genotypes isolated from animals have never been isolated from human. Only genotype *bca*$^+$ *scpB*$^+$ *bac* were found to be specific for both bovine and human strains. It probably means that only GBS strains of *bca*$^+$ *scpB*$^+$ *bac* genetic type can be exchanged between animals and human and considered as a causative agent for both animals and human. Meanwhile this proposal should be confirmed in the model experiments.

In conclusion, gene *bac*, encoding IgA binding β antigen, was not found among bovine GBS strains. Human GBS strains, carrying *bac* gene, were characterized by almost identical complex hybridization patterns with multiple gene probes. Gene *scpB*, encoding C5a peptidase in all human GBS strains was found in 23 % of bovine GBS strains. Among 5 genetic types discovered for GBS strains under study only strains of *bca*$^+$ *scpB*$^+$ *bac* genotype can be probably exchanged between animals and human and considered as a causative agent both for animals and human.

REFERENCES

BAKER C.J., EDWARDS M.S.: Group B streptococcal infections, pp. 742–811 in I.S. Remington, J.O. Klein (Eds): *Infectious Diseases of the Fetus and Newborn Infant.* Saunders, Philadelphia (PA) 1990.

BENTLEY R.W., LEIGH J.A.: Determination of 16S ribosomal RNA gene copy number in *Streptococcus uberis, S. agalactiae, S. dysgalactiae* and *S. parauberis. FEMS Immunol.Med.Microbiol.* **12**, 1–7 (1996).

CHMOURYGUINA I.I., SUVOROV A.N., FERRIERI P., CLEARY P.P.: Conservation of C5a peptidase genes in group A and B streptococci. *Infect.Immun.* **64**, 2387–2390 (1996).

DENNING D.V., BACER C.J., TROUP N.J., TOMPKINS L.S.: Restriction endonucleases analysis of human and bovine group B streptococcus for epidemiologic study. *J.Clin.Microbiol.* **27**, 1352–1356 (1989).

DMITRIEV A., SUVOROV A., TOTOLIAN A.: Physical and genetic chromosomal maps of *Streptococcus agalactiae*, serotypes II and III; rRNA operon organization. *FEMS Microbiol.Lett.* **167**, 33–39 (1998).

FORSMAN P., TILSALA-TIMISJARVI A., ALATOSSAVA T.: Identification of staphylococcal and streptococcal causes of bovine mastitis using 16S–23S rRNA spacer regions. *Microbiol.Read.* **143**, 3491–3500 (1997).

HEDEN L.O., FRITHZ E., LINDAHL G.: Molecular characterization of an IgA receptor from group B streptococci: sequence of the gene, identification of a proline-rich region with unique structure and isolation of N-terminal fragments with IgA-binding capacity. *Eur.J.Immunol.* **21**, 1481–1490 (1991).

JERLSTROM P.J., CHHATWAL G.S., TIMMIS K.N.: The IgA binding *b* antigen of the C protein complex of group B streptococci: sequence determination of its gene and detection of two binding regions. *Mol.Microbiol.* **5**, 843–849 (1991).

MANIATIS T., FRITSCH E.F., SAMBROOK J.: *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY) 1982.

MICHEL J.L., MADOFF L.C., OLSON K., KLING D.E., KASPER D.L.: Large, identical tandem repeating units in the C protein α antigen gene, *bca*, of group B streptococci. *Proc.Nat.Acad.Sci.USA* **89**, 10060–10064 (1993).

SMOLA J.: Pig and nutria as hosts of *Streptococcus agalactiae*, p. 121 in *Abstr. 12th Lancefield Internat. Symp. Streptococci and Streptococcal Diseases*, Saint Petersburg (Russia) 1993.

SUVOROV A., DMITRIEV A., USTINOVICH I., SCHALEN C., TOTOLIAN A.: Molecular analysis of clinical group B streptococcal strains by use of α and β gene probes. *FEMS Immunol.Med.Microbiol.* **17**, 149–154 (1997).

KOŠICE
St. Elizabeth's Cathedral

Cold water geyser
in Herľany (near Košice)